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DEVELOPING HIGH OLEIC ACID FLAX OIL

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DEVELOPING HIGH OLEIC ACID FLAX OIL

ADF project # 99000099

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Abstract:

Seeds from nearly 1100 mutagenized field grown plants (solin breeding line S95407) were screened for fatty acid composition. Twelve plants were identified as having elevated levels of oleic acid in the seed oil from the EMS treated plants. None of the irradiated plants showed any substantial alteration in fatty acid levels. Elevated levels were determined as plants in which 2 or more seeds displayed oleic acid levels greater than 34% of total fatty acids. A number of mutants have now been bred to the M4 generation. All lines showed stable oleic acid content (40 – 50%) through he M3 generation. Crosses were also made between the mutants and several F6 solin lines that had been selected in field studies as having higher than normal oleic acid levels (30 – 35%). Finally a couple of crosses were between the oleic acid mutants and a transgenic liner with elevated oleic acid. Analysis of seed from majority of these cross strategies showed some increase in the total oleic acid. However, regardless of the cross the maximum oleic acid content observed was between 55- 60%. Most of this material will be ready to plant in the field during the 2002 crop season to evaluate oleic acid levels under field conditions,. Lastly a mutant was identified as having elevated stearic acid (C18:0) content (10% compared to 3% in control lines). This line was crossed with a high palmitic acid containing solin and resulting progeny were found to have enhanced levels of both palmitic and stearic acid.

EXECUTIVE SUMMARY

- Seeds of the solin breeding line S95407 were mutagenized using either ethylmethanesulphonate (EMS) treatment or gamma irradiation.
- Mutagenized lines were grown at Kernen Research Farm in the summers of 1999 and 2000 and individually harvested and threshed.
- Gas chromatography was used to analyze the fatty acid composition of 738 EMS treated lines and 360 irradiated lines.
- Twelve lines were identified as having elevated oleic acid composition (34% or greater oleic acid)
- One line was found to have elevated levels of stearic acid
- Crosses were made between various oleic acid mutants, oleic acid mutants and breeding material with elevated oleic acid (25 – 30%) and a transgenic line showing increased oleic acid.
- Anther culture generated an oleic acid mutant which has been used in several crosses.
- A large number of crosses showed complementation of the mutations and were developed to the M4 generation.
- Analysis of the seed oil of the various crosses showed an enhanced level of oleic acid in many of the lines, however no line developed had oleic acid levels greater than 60%,
- A cross with the stearic acid mutant and a high palmitic acid solin showed increased levels of both palmitic acid (40%) and stearic acid (15%).

INTRODUCTION:

Flax has traditionally been grown as an industrial oilseed, although there has been an increasing use of flaxseed in confectionery and bakery goods. However, the introduction of the low linolenic acid containing flax (solin) with an oil type similar to sunflower will allow for increased utilization of flax seed in the human edible oil market. These solin varieties were created through mutagenesis programs at various research institutes including the Crop Development Centre.

A trend that is becoming prevalent in that oilseed industry is the creation of varieties that have specific fatty acid profiles that are tailored to the projected end of the oil. One fatty acid type that has been high on the objective list in every oilseed is oleic acid content. Both canola and soybean varieties have been created that contain greater than 80% oleic acid. The soybean varieties were created through genetic engineering while the canola variety was created through a mutagenesis program.

The CDC created 4000 lines of EMS mutagenized flaxseed and this collection has been screened for a number of traits including fatty acid mutations. To date several lines have been identified that show low linolenic acid levels and one line was found that contained elevated levels of palmitic acid. However, no significant changes were found in oleic acid content in any of the lines that were screened by gas chromatography. It should be noted that only lines that were initially identified as having a reduced level of linolenic acid by the TBA test were selected for screening by GC. We believed that it was reasonable to expect that a flax line that has a mutant $\Delta 12$ desaturase gene showing enhanced oleic acid levels in the seed oil. This desaturase converts oleic acid to linoleic acid.

Finally, an advantage to using the EMS approach, rather than a genetic engineering approach is the ready acceptance of varieties produced through mutagenesis programs compared to the regulatory hurdles required for the registration of genetically engineered varieties.

Materials and Methods:

Seeds of the solin line S95417 were soaked in 0.8% EMS at room temperature for 4 hours and then rinsed overnight at 4°C with a constant stream of water. Seeds were dried to ambient humidity levels and seeded at Kernen Research Farm. Plants were individually harvested and hand threshed. Additional seeds of S95407 were sent to India for treatment by gamma irradiation. Seeds were grown in growth cabinets during the winter of 1999/2000 to determine which dosage was likely to be the most effective treatment. The irradiated seeds were then grown at Kernen Research Farm during the 2000 season. Plants were individually harvested and seeds are stored for future analysis.

Fifteen seeds from each plant were initially screened by GC. Plants that indicated an elevation in oleic acid were rescreened by half seed analysis. Half seed analyses were conducted by cutting the cotyledon of ten seeds from each selected plant. The remaining portion of the seed was placed in germination medium and allowed to germinate during the fatty acid analysis. The excised half seed was then analyzed for fatty acid composition by GC Seedlings that were identified as having the elevated oleic acid were transferred to pots with Ready Earth mixture and raised to maturity. Crosses have been made between various mutants and between mutants and other flax and solin material. Finally, anther culture was conducted on the F1 plants of a solin x flax cross and the resulting doubled haploid plants were analyzed for fatty acid composition.

Fatty acid composition was determined by gas chromatography. Single seeds were randomly selected from the various plots and baked @ 100°C for 1 h in a methanol solution containing 2% sulfuric acid, and then extracted with hexane. The hexane was injected into a J&W DB-23 column installed in an HP 5890 Series II GC using an isothermic temperature gradient (220°C) and helium @ a flow rate of 1 ml min-1 as a carrier gas. Fatty acid identification was based on retention times of known standards.

Results:

Twelve of the nearly 1000 mutant lines were identified as having potential mutations in one of the Δ12 desaturase genes present in flax. The lines displayed oleic acids levels of at least 34% in two or more of the 15 seeds selected seeds from each plant. The maximum oleic acid level that was observed in half seed selections was 46%. These levels compare to the average oleic acid content of 15 – 20% in non-mutagenized control plants grown under the same conditions. Half seed selections were completed on all the selected lines. It is important to note that these oleic acid levels were from field grown material as selections from growth chambers are not stable for this trait.

Seed from M2 plants from the 11 of the original 12 selected lines showed elevated oleic acid content. These lines were all raised to the M3 generation, confirmed for homozygousity and used in a variety of crosses. Little significant difference was noted in the oleic acid levels in when the heterozygous and homozygous mutants although raising the material in growth cabinets leads to greater fluctuations in fatty acid composition than field conditions. Homozygous plants from the eleven putative mutants have been used in crosses amongst themselves in an attempt to complement the mutations to further enhance the oleic acid levels.

Ten seeds from the crosses were subjected to half seed analysis and selected lines were then grown to maturity and selfed. In several crosses no increase in the oleic acid content was noted. This was not unexpected, and merely indicates that the crosses were between plants harboring the same mutation. In many of the crosses an increase of 10 –15% in the oleic acid was noted suggesting that two defective Δ12 desaturase genes were present in the germplasm. None of the crosses ever resulted in oleic acid contents greater than 60%. This is in contrast the work with the Δ15-desaturase mutations in the development of the original solin varieties, where the activity of this enzyme was effectively eliminated with mutations to two genes. This is evidence for at least a third Δ12-desaturase gene that is functional in the seed. A series of three way crosses were then initiated amongst the mutants, however no further increase in the oleic acid content was noted.

The EMS mutants were also used to make crosses with selections of field grown solin lines that showed slightly elevated oleic acid content. The exact mechanism resulting in enhanced oleic acid in these lines is unknown. Similar to the results observed with the crosses between to the various mutants, an increase in the seed oleic acid content was noted in the majority of the progeny. However, the maximum level of oleic acid achieved was 50%, which was less than had been achieved with the mutants crosses.

An unexpected source of oleic acid germplasm was identified during the timeframe of this project. Within the anther culture program crosses were made between various solin and flax breeding lines. Fatty acid analysis revealed one doubled haploid plant to have altered oleic acid content (~50%). This plant likely arose due to a process called somaclonal variation – spontaneous mutations generated while in tissue culture. This line was crossed with two distinctly different EMS mutant lines. In each case, an increase in the total oleic acid was observed in the progeny, but once again a maximum level of 60% was found.

Finally, with respect to oleic acid composition, a cross was made with a transgenic flax line expressing a soybean phosphotidylcholine acyl transferase gene. This line had shown increased oleic acid levels (~40%). This mechanism of increasing the oleic acid content differs from the elimination of the endogenous desaturase activity that is responsible for the EMS fatty acid changes. It was thought that these two different mechanisms may result in overcoming the 60% barrier we had encountered in other attempts. The progeny of this cross did not show an increase in the overall level of oleic acid although an intermediate level of linolenic acid and linoleic acid were noted. (The flax transgenic line was not a solin type).

A stearic (C18:0) acid mutant was also noted while screening the mutant germplasm. Stearic acid has value in the confectionery industry and as a margarine component. A line with 10% stearic was identified and breeding to homozygousity, was crossed with a high palmitic acid (25% palmitic acid) solin line. The resulting progeny showed elevated level

of both palmitic acid (35- 42%) and stearic acid (15%). This material is being advanced as a possible industrial oil feedstock line.

Five of the oleic acid mutant lines were grown at Kernen Research Farm during the summer of 2001. We were able to recover our seed from this material, but have not analyzed this material for fatty acid composition. Given the growing conditions this past summer, we are not entirely sure how valid the data we will obtain is going to be. It is going to have to be compared to with a number of other flax/solin types where the fatty acid composition is well documented.

In conclusion, we have been able to generate stable germplasm with an oleic acid level of nearly 60%. This level of oleic acid is comparable to several other oilseeds, but did not reach the 70 – 80% content that has been achieved in canola, soybean and sunflower. A possible reason for our inability to achieve a greater oleic acid content is that is may be that a third (or fourth) Δ12-desaturase gene exists in the flax/solin genome and may be absolutely required for plant development (i.e. plasma membrane synthesis). Earlier work at the Plant Biotechnology Institute and the Crop Development Centre, found that the Δ9-desaturase genes in flax are expressed in both the vegetative and seed tissues. One copy was preferentially expressed in the seed while the other was expressed in both seed and leaves. Similar control of a Δ12-desaturase gene could be responsible for the residual Δ12-desaturase activity we are unable to eliminate. If this gene is expressed in both the vegetative and seed tissues, and is essential for plant development, the only way to further increase oleic acid content would be using a transgenic approach that specifically targeted the seed expression.